Thermally-induced Changes in Poly-α-amino Acids Containing Methyl Sidechains[1]

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Poly-α-amino acids containing α-methyl sidechains have been synthesized by the Leuchs method. These acids include poly-L-alanine, poly-DL-alanine, poly-α-aminoisobutyric acid, copoly(DL-alanine, L-glutamic acid) and copoly(α-aminoisobutyric acid, L-glutamic acid). Copolymers have been coagulated in an aqueous solution by heat; other changes in the properties of both polymers and copolymers have been studied by the methods or measurements of specific rotation, specific viscosity, differential thermal analysis and infrared absorption spectra. The effects have been interpreted as being due to changes in the secondary structure. The poly-α-amino acids containing the alanine residue show changes in these properties at about 60°C, while the polymers containing the α-aminoisobutyric acid residue show such changes at about 50°C. The infrared absorption spectra indicate a change from the α to the β form.

It has been reported that poly-ε-aminocaproyl-DL-alanine,[2] poly-ε-aminocaproyl-α-aminoisobutyric acid[3] and poly-ε-aminocaproyl-L-proline[4] are slightly soluble in water and that these solutions are reversibly coagulated by heat; they precipitate from an aqueous solution upon being heated and redissolve upon being cooled. Kurtz et al.[5] observed the same phenomenon with poly-L-proline. Elliott[6] reported the thermal conversion of poly-DL-alanine from the α to the β formin an aqeuous solution. The β-form can be converted into the α-form by treating it with formic acid.[6]

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[1] Presented at the 10th Annual Meeting of the Society of Polymer Science Japan, Tokyo, May, 1961.

[2] J. Noguchi, T. Hayakawa, J. Suzuki and M. Ebata, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **76**, 648 (1955); J. Noguchi, T. Hayakawa and M. Ebata, *J. Polymer Sci.*, **23**, 843 (1955).

[3] J. Noguchi, M. Ebata, T. Hayakawa and T. Saito, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **77**, 469 (1956).

[4] J. Noguchi et al., *Annual Report of the Fiber Research Institute, Japan*, **9**, 118 (1956).

[5] J. Kurtz, A. Berger and E. Katchalski "Recent Advances in Gelatin and Glue Research," Ed. by G. Stanish, Pergamon Press, London (1958), p. 131.

[6] A. Elliott, *Nature*, **170**, 1066 (1952); C. H. Bamford, L. Brown, A. E. Elliott, W. Hanby and I. F. Trotter, ibid., **173**, 27 (1954).

TABLE I. MOLAR RATIOS OF REACTANTS AND SOLUBILITIES OF POLY-α-AMINO ACIDS

No.	Polymer	Mole ratio	Solubility in water
I	Poly-L-alanine		Insoluble
II	Poly-DL-alanine		Easily soluble
III	Poly-α-aminoisobutyric acid		Insoluble
IV-1	Copoly(DL-alanine, L-glutamic acid)	1 : 1	Insoluble
IV-2	Copoly(DL-alanine, L-glutamic acid)	2 : 1	Insoluble
IV-3	Copoly(DL-alanine, L-glutamic acid)	5 : 1	Slightly soluble
IV-4	Copoly(DL-alanine, L-glutamic acid)	10 : 1	Slightly soluble
IV-5	Copoly(DL-alanine, L-glutamic acid)	20 : 1	Soluble
V-1	Copoly(α-aminoisobutyric acid, L-glutamic acid)	1 : 1	Insoluble
V-2	Copoly(α-aminoisobutyric acid, L-glutamic acid)	2 : 1	Insoluble
V-3	Copoly(α-aminoisobutyric acid, L-glutamic acid)	5 : 1	Slightly soluble
V-4	Copoly(α-aminoisobutyric acid, L-glutamic acid)	10 : 1	Slightly soluble
V-5	Copoly(α-aminoisobutyric acid, L-glutamic acid)	20 : 1	Slightly soluble

Methyl groups of alanine or α-aminoisobutyric residues and tetrahydropyrrole rings of proline residues may contribute to the alteration of the secondary structure of the polypeptide chain upon being heated possibly resulting in the coagulation phenomenon. These groups may also be important in the heat denaturation of protein. This paper reports on the effect of heat upon various properties of poly-α-amino acids containing α-methyl groups. The polymers studied were poly-L-alanine (I), poly-DL-alanine (II), poly-α-aminoisobutyric acid (III), copoly(DL-alanine, L-glutamic acid) (IV), and copoly(α-aminoisobutyric acid, L-glutamic acid) (V). They were prepared by the Leuchs method⁷⁾ (Table I), glutamic acid being protected as the γ-benzyl ester during the synthesis.

The properties of the polymers studied, or the methods of studying the reversible heat coagulation, were specific rotation, specific viscosity, differential thermal analysis and infrared absorption spectra.

Experimental

The polymers studied and their solubilities in water are listed in Table I.

Poly-L-alanine (I).—This was prepared by the usual method.⁷⁾ The L-alanine was suspended in dry dioxane and treated with dry phosgene, and the resulting Leuchs anhydride was polymerized n benzene at 80°C for 48 hr. The polymer was nsoluble in all the solvents tested except dichloroacetic acid. The intrinsic viscosity was measured with an Ostwald viscosimeter in dichloroacetic acid at 30±0.01°C. $[\eta]=[\eta_{sv/c}]_{c\to 0}=0.0216$, ml./g.

Poly-DL-alanine (II).—This was prepared in the same way as was poly-L-alanine. The molecular weight was determined by *N*-terminal analysis by titration with perchloric acid in glacial acetic acid. Mol. wt. = 5000.

Poly-α-aminoisobutyric Acid⁸⁾ (III).—α-Aminoisobutyric acid (6.0 g.) was suspended in 300 ml. of dry dioxane, and dry phosgene was passed in at 50°C. It was almost completely dissolved after ten hours. Carbon dioxide was then passed in to remove the excess phosgene, and the solvent was removed under reduced pressure. The residual crystals were treated with petroleum ether, filtered and dried. The Leuchs anhydride was recrystallized from ethyl acetate-petroleum ether; yield, 5.0 g. The Leuchs anhydride (5.0 g.) was dissolved in 100 ml. of dry dioxane and polymerized with a sodium methylate initiator at room temperature. After a week, the solvent was removed, and the residue was treated with water, filtered and dried; yield, 2.2 g. (66.7%).

This polymer was insoluble in all the solvents tested except dichloroacetic acid. The intrinsic viscosity was measured in dichloroacetic acid in the same way as was the poly-L-alanine at 30±0.01°C. $[\eta]=[\eta_{sp/c}]_{c\to 0}=0.00187$, ml./g.

Copoly(DL-alanine, L-glutamic acid) (VI).—The method of preparing IV-2, IV-3, IV-4, and IV-5 samples has been reported earlier.⁸⁾ The IV-1 sample was prepared in the same way.

Found: N, 14.00; Calcd. for $1C_3H_5ON$-$1C_5H_7O_3N$: N, 14.00%.

The molecular weight was determined by the *N*-terminal titration of the intermediate, copoly(DL-alanine, γ-benzyl-L-glutamate); mol. wt. = 19000.

Copoly(α-aminoisobutyric acid, L-glutamic acid) (V).—Mixtures of α-aminoisobutyric acid NCA and γ-benzyl-L-glutamate NCA were each dissolved in dry dioxane in the ratios of 1 : 1, 2 : 1, 5 : 1, 10 : 1 and 20 : 1. Each mixture was polymerized for several

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7) H. Leuchs, *Ber.*, **39**, 857 (1906); A. C. Farthing and R. J. W. Reynolds, *Nature*, **165**, 647 (1950).

8) J. Noguchi, *High Polymer Chem., Japan* (*Kobunshi Kagaku Zassi*), **6**, 193 (1949).

TABLE II. COPOLYMERS OF α-AMINOISOBUTYRIC ACID AND γ-BENZYL L-GLUTAMATE

Copolymer	Amino acid Mole ratio	NCA g.	Initiator mg.	Yield g.	Amino acid composition Mole ratio	Mol. wt.	N, % Found	N, % Calcd.
α-Aminoisobutyric acid	1	3.58	15.1	7.0	1	9700	9.10	9.21
γ-Benzyl L-glutamate	1	7.30			1			
α-Aminoisobutyric acid	2	5.39	17.0	5.5	2	5500	10.60	10.79
γ-Benzyl L-glutamate	1	5.50			1			
α-Aminoisobutyric acid	5	8.08	20.2	5.1	4.9	6100	12.85	13.00
γ-Benzyl L-glutamate	1	3.30			1			
α-Aminoisobutyric acid	10	9.82	23.0	5.1	9.9	5400	14.02	14.20
γ-Benzyl L-glutamate	1	2.00			1			
α-Aminoisobutyric acid	20	11.4	25.0	5.6	19	4100	14.98	15.27
γ-Benzyl L-glutamate	1	1.15			1			

days at room temperature, using sodium methylate as the initiator. The mixture in each case was then heated on a boiling water bath for one hour, and the solvent was removed under reduced pressure. The residue was treated with much water, filtered, and dried. The molecular weight was determined by endgroup titration. Portions of these copolymers were hydrolyzed with 6 N hydrochloric acid, and the component amino acids were assayed from paper chromatograms after ninhydrin staining by means of the densitometer (Table II).

These copolymer were debenzylated with glacial acetic acid and hydrogen bromide at 50°C in the usual way[10] (Table III).

TABLE III. COPOLYMERS OF α-AMINOISOBUTYRIC ACID AND L-GLUTAMIC ACID

		N, %	
	Mole ratio	Found	Calcd.
α-Aminoisobutyric acid	1	12.92	13.08
L-Glutamic acid	1		
α-Aminoisobutyric acid	2	13.70	14.05
L-Glutamic acid	1		
α-Aminoisobutyric acid	5	14.84	15.14
L-Glutamic acid	1		
α-Aminoisobutyric acid	10	15.47	15.72
L-Glutamic acid	1		
α-Aminoisobutyric acid	20	15.66	16.06
L-Glutamic acid	1		

Discussion

Reversible Heat Coagulation.—It was not possible to test the water-insoluble poly-L-alanine (I), poly-α-aminoisobutyric acid (III), copoly(DL-alanine, L-glutamic acid) (IV) (1)–(2) and copoly(α-aminoisobutyric acid, L-glutamic acid) (V) (1)-(2) for reversible coagulation by heat. The water-soluble poly-DL-alanine and copoly(DL-alanine, L-glutamic acid) (V-5) were also not coagulated by heat, possibly because of their high solubility in water. Other slightly-soluble copolymers, IV-3, IV-4, V-3, V-4 and V-5, were reversibly coagulated by heating and cooling. The relationship between the turbidity and the temperature is shown in Figs. 1 and 2. The turbidity increased with an increase in the temperature, the polymers of group IV, which contains DL-alanine, reaching a constant value at about 60°C, and those of groups V, which contains α-aminoisobutyric acid, reaching one at about 50°C. However, the ascending and descending temperature curves did not coincide. When the glutamic acid residues were converted to sodium salts, the water solubility of these

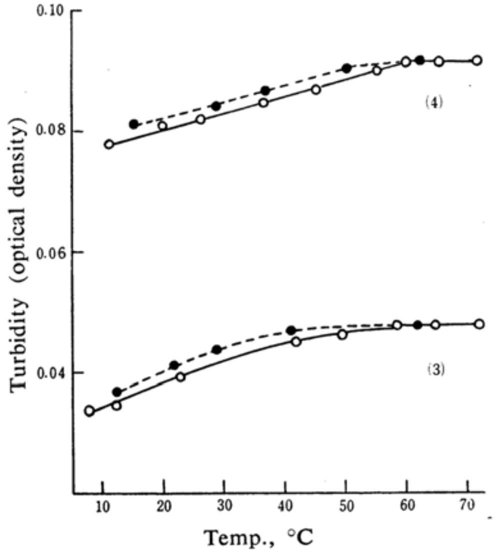

Fig. 1. Turbidity of copoly(DL-ala, L-glu) IV concentration: IV-3 0.45%, IV-4 0.62%.
(Measured after keeping for 15 min. at each temp.)
○—○ Points obtained with rising temp.
●—● Points obtained with falling temp.

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9) J. Noguchi, T. Saito, T. Hayakawa, H. Tokuyama and T. Harada, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **82**, 597 (1961).

10) D. Ben-Ishai, *J. Org. Chem.*, **19**, 62 (1954).

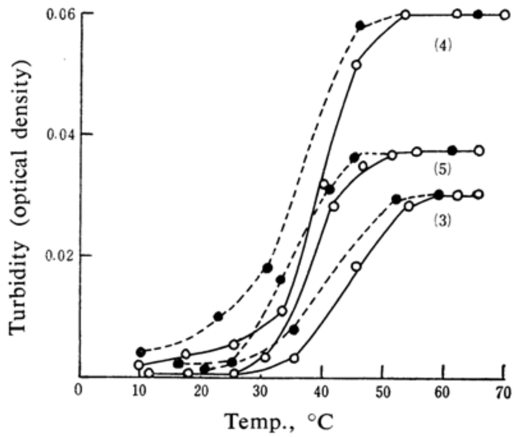

Fig. 2. Turbidity of copoly(α-aminoisobut., L-glu) V concentration: V-3 1.8%, V-4 2.1%, V-5 2.7%.
(Measured after keeping for 15 min. at each temp.)
○—○ Points obtained with rising temp,
●—● Points obtained with falling temp.

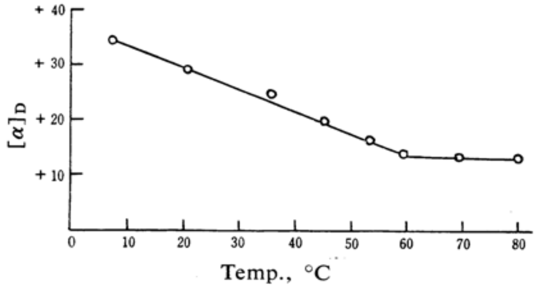

Fig. 3. Optical rotation of poly-L-alanine I ($c=1.31$, dichloroacetic acid).
(Measured after keeping for 15 min. at each temp.)

samples increased, and they were no longer heat-coagulable.

Specific Rotation.—The effect of the temperature on the optical rotation of these polymers is shown in Figs. 3, 4 and 5. The levorotation of poly-L-alanine (I) (Fig. 3), measured in dichloroacetic acid, decreased with a rise in the temperature, reaching, however, an almost constant value at about 60°C. The sodium salt of copoly IV also exhibited the same critical temperature, as Fig. 4 shows. The sodium salt of copolymer V had a critical temperature of about 50°C, as is shown in Fig. 5. These optical properties are complementary with the reversible heat coagulation phenomena. It is possible that the structure of the polymer chain which has methyl side-chains may be altered by heating, and that this may cause delicate changes in the solubility. The poly-α-amino acids containing the alalyl residue seem to undergo a change (or changes) in their secondary structure at about 60°C, while the polymers containing α-aminoisobutyryl residues show their initial response at about 50°C.

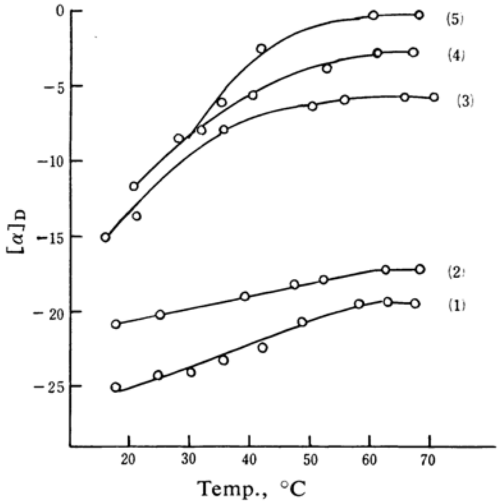

Fig. 4. Optical rotation of copoly(DL-ala, L-glu-Na) IV in water (1) $C=5.00$, (2) $c=5.00$, (3) $c=5.00$, (4) $c=3.46$, (50) $c=1.71$.
(Measured after keeping for 15 min. at each temp.)

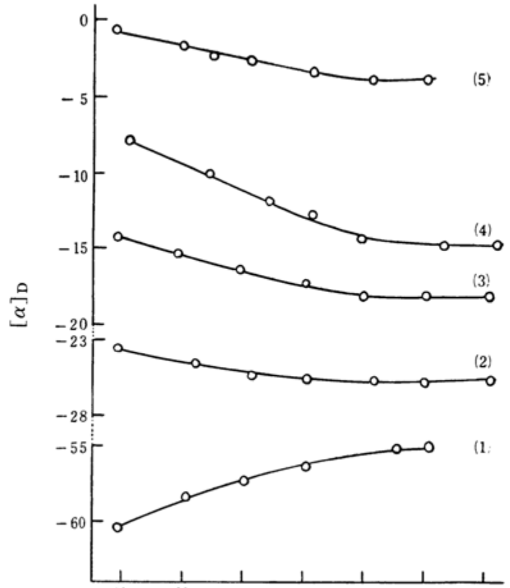

Fig. 5. Optical rotation of copoly(α-aminoisobut, L-glu-Na) V in water (1) $c=1.85$, (2) $c=3.98$, (3) $c=1.14$, (4) $c=2.30$, (5) $c=1.50$.
(Measured after keeping for 15 min. at each temp.)

In order to verify these phenomena, specific viscosity determinations, differential thermal analyses and infrared spectra were performed on poly-L-alanine (I), poly-DL-alanine (II), and poly-α-aminoisobutyric acid (III).

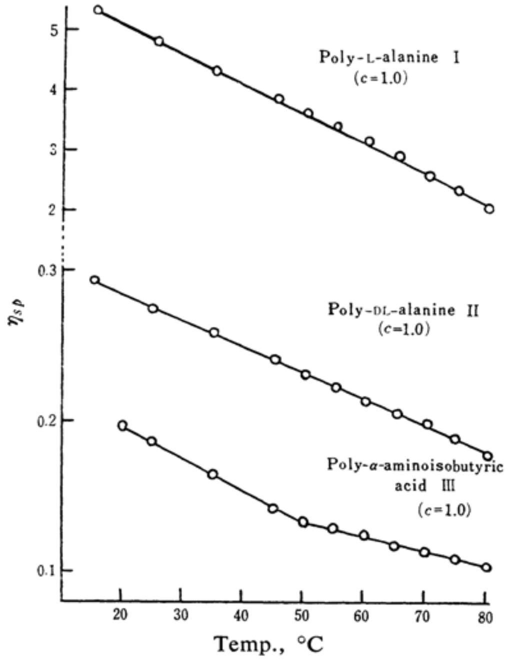

Fig. 6. Specific viscosity in dichloroacetic acid.
(Measured after keeping for 30 min. at each temp.)

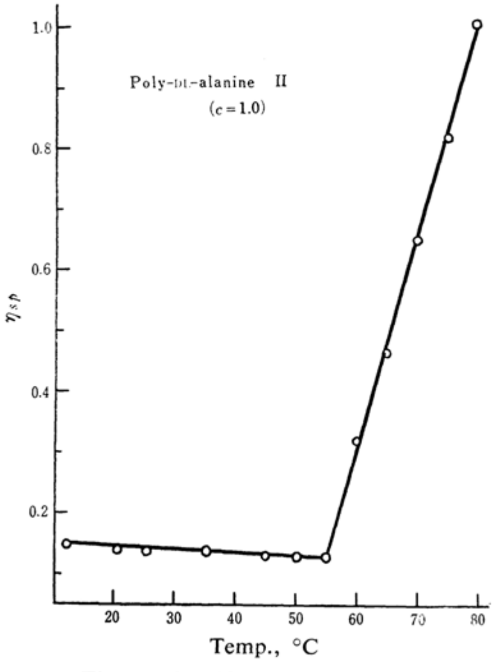

Fig. 7. Specific viscosity in water.
(Measured after keeping for 2 hrs. at each temp.)

Specific Viscosity.—The specific viscosities of I, II and III in 1% dichloroacetic acid solutions were measured in an Ostwald viscosimeter at varying temperatures, as is shown in Fig. 6. The specific viscosity decreased with a rise in the temperature. The viscosity of I and II decreased linearly, while the viscosity of III was observed to have a transition point at 50°C. In a dichloroacetic acid solution, the change in viscosity was not great. II was water-soluble; the specific viscosity in an aqueous solution is shown in Fig 7. The measurements of viscosity were carried out after the substance had stood for two hours at each temperature. In this case, the specific viscosity decreased slightly until the temperature reached 55°C but it increased rapidly when the temperature exceeded 55°C. This finding is consistent with Ebata's report,[11] in which the aqueous solution of poly-ε-aminocapryol-DL-alanine showed a minimum viscosity at about 55°C, with an increase at higher temperatures. This effect may be due to the influence of alanine residues in the polymer. The aqueous solution of II at more than a 4% concentration formed a gel at 60°C or a higher temperature, as has been reported by Elliott.[6]

Differential Thermal Analyses.—The differential thermal analytical data for I, II and III are shown in Fig. 8. With polymer I, an endothermal change was observed from 38 to 120°C, with polymer II, from 60 to 160°C, and with polymer III, from 50 to 120°C. The

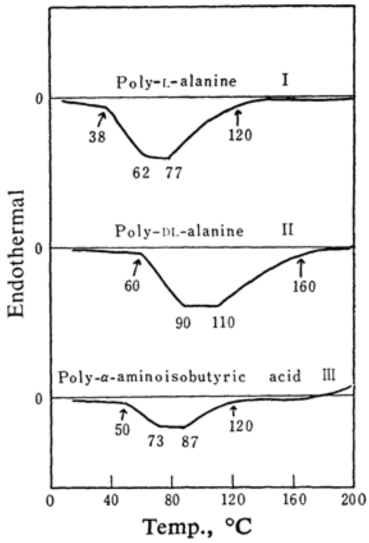

Fig. 8. Differential thermal analyses.

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[11] M. Ebata, This Bulletin, 33, 899 (1960).

maximum endothermal change was observed from 62 to 77°C, from 90 to 110°C and from 73 to 87°C, respectively, for the polymers I, II and III. The endothermal changes of poly-DL-alanine (II) and poly-α-aminoisobutyric acid (III) started at 60 and 50°C respectively, they are in good agreement with the viscosity and heat coagulation curves.

Infrared Analyses.—The infrared absorption spectra of I, II and III that were measured at room temperature were compared with the spectra that were measured at the maximum endothermal value temperature in the differential thermal analyses, i. e., at 62°C for I, at 100°C for II and at 80°C for III (Fig. 9). The observations were carried out after two hours at the above-mentioned temperatures. The dotted lines in Fig. 9 depict the spectra observed at higher temperatures, which are different from those measured at room temperature. At high temperatures, poly-L-alanine (I) (film) showed a wider absorption at 3300 cm^{-1}, (νNH) and a broader absorption at about 2070 cm^{-1} than the spectra measured at room temperature. In a region longer than 1000 cm^{-1}, the absorption spectra showed a different pattern, which may be due to the conformational changes in the polymer. These differences observed in the absorption spectra disappeared when the sample was cooled to room temperature. The absorption bands at 1660 cm^{-1} (Amide I) and at 1550 cm^{-1} (Amide II) appeared slightly broader when they were measured at a higher temperature. This effect may be due to the shift from the α to the β form. These phenomena were observed more clearly with poly-DL-alanine (II) (film) and poly-α-aminoisobutyric acid (III) (pellet). The absorption bands at 1630 cm^{-1} and at 1530 cm^{-1} in II were relatively weak at room temperature, but they were significantly stronger at 100°C. This behavior may be explained by the shift from the α to the β conformation of the peptide chain. With III, only the 1660 cm^{-1} and 1550 cm^{-1} bands were exhibited at room temperature. At higher temperatures, however, these bands were split, the former into 1660 cm^{-1} and 1630 cm^{-1} and the latter into 1550 cm^{-1} and 1530 cm^{-1}. This might represent a shift to the β-form from the α-form, as has been postulated above. The absorption due to methyl at 2960 cm^{-1} in I and II was strong at a high temperature, and the absorption with III, at about 3000 cm^{-1} was very strong because of the additional methyl group. Again, a conformational change from the α-form to the β-form is assumed to occur at a high temperature.

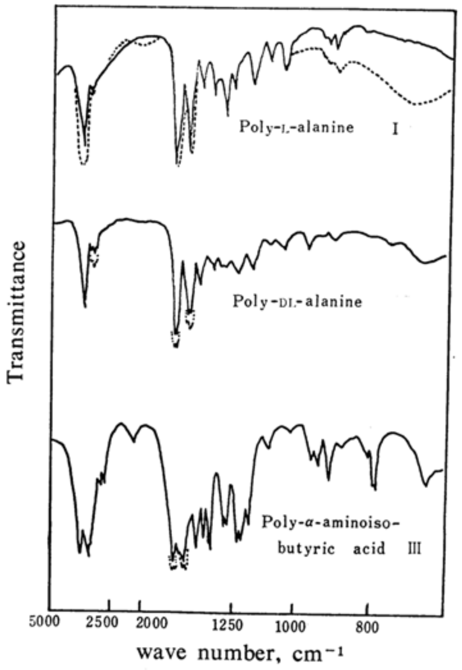

Fig. 9. Infrared absorption spectra.

Conclusion

Poly-α-amino acids containing alanine and α-aminoisobutyric acid, i. e., containing one and two α-methyl groups respectively, have been synthesized. From the findings of coagulation phenomena, specific rotation, specific viscosity and differential thermal analysis, these poly-α-amino acids containing the alanine residue and the α-aminoisobutyric acid residue may be understood to undergo steric structural changes at about 60°C and 50°C respectively. The difference in critical temperature between the above two polymers can be explained by the presence of one more methyl group per residue in the polymer from α-aminoisobutyric acid. From the infrared results a conformational change at high temperature from the α- to the β-form has been inferred.

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